Safety Data Sheet

Aflatoxins B_1, B_2, G_1, G_2, M_1, M_2, and P_1

Division of Safety
National Institutes
of Health

WARNING!

THESE COMPOUNDS MAY BE ABSORBED THROUGH THE RESPIRATORY AND INTESTINAL TRACTS. THEY MAY BE TOXIC, CARCINOGENIC, MUTAGENIC, AND TERATOGENIC. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND WATER.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK WATER. INDUCE VOMITING. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. WASH DOWN AREA WITH HYPOCHLORITE SOLUTION, FOLLOWED BY 5% AQUEOUS ACETONE. SEE IARC (1980) FOR DETAILS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

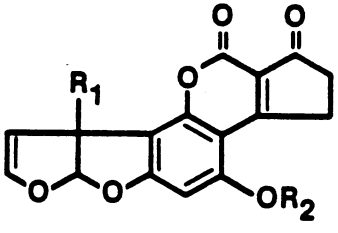
Aflatoxins (AF) are a class of naturally occurring compounds, produced by strains of the mold Aspergillus flavus and related species and distributed on a wide variety of foodstuffs. In pure form, they are colorless to pale yellow crystals with intense fluorescence in ultraviolet light, unstable on exposure to light and air. The term "aflatoxin" has been applied to both the natural products and some of their metabolites that retain the basic ring structure. The natural aflatoxins are divided into two classes of two compounds each on the basis of their emission,

issued 10/82

under ultraviolet light, of a blue (AFB_1, AFB_2) or green (AFG_1, AFG_2) fluorescence.* They are highly toxic in rodents, highly carcinogenic in rodents and primates, mutagenic in the Ames test, and (where data are available) teratogenic. Their use is limited to research on carcinogenicity.

B. Chemical and Physical Data

Note: The position assignments in the aflatoxin ring structure are not always consistent. The chemical names given below are based on Chemical Abstract use; in this system the double-bonded carbons of th first (left) furan ring are assigned positions 8 and 9. However, all literature reports on oxidation products (metabolites) such as the epoxide and the dihydrodiol refer to these positions as 2 and 3. To confuse the issue further, aflatoxin Q_1 is designated as 3-hydroxy-aflatoxin B_1 but in this case position 3 is consistent with Chemical Abstract nomenclature and refers to the carbon atom in meta position to the ketone group in the last (cyclopentenone) ring.



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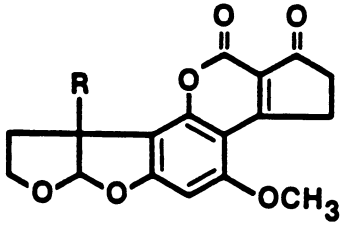

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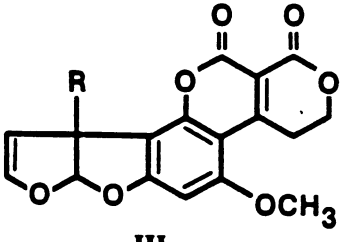

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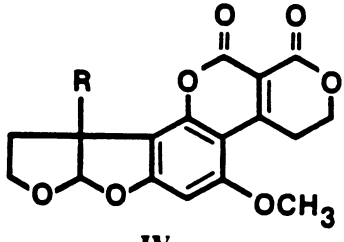

IV

Individual Chemical and Physical Data

Aflatoxin B_1

1. Chemical Abstract No.: 1162-65-8

Actually, this is true for AFG_1 as normally purified because of a yellow impurity. Very highly purified AFG_1 shows blue fluorescence (Lijinsky and Butler, 1966). The same may also apply to AFG_2, though there are no data.

. Synonyms:

AFB_1 Aflatoxin B

Cyclopenta(c)furo(3',2':4,5)furo(2,3-h)(1)benzopyran-1,11-dione, 2,3,6a,9a-tetrahydro-4-methoxy- (9CI)

. Molecular formula: $C_{17}H_{12}O_6$

structure: I, $R_1 = H$, $R_2 = CH_3$

weight: 312.29

flatoxin B_2

Chemical Abstract No.: 7220-81-7

Synonyms:

AFB_2 Dihydroaflatoxin B_1

Cyclopenta(c)furo(3',2':4,5)furo(2,3-h)(1)benzopyran-1,11-dione, 2,3,6a,8,9,9a-hexahydro-4-methoxy- (9CI)

Molecular formula: $C_{17}H_{14}O_7$

structure: II, R = H

weight: 314.31

latoxin G_1

Chemical Abstract No.: 1165-39-5

Synonyms:

AFG_1

1H,12H-Furo(3',2':4,5)furo(2,3-h)pyrano(3,4-c)(1)benzopyran-1,12-dione, 3,4,7a,10a-tetrahydro-5-methoxy- (9CI)

Molecular formula: $C_{17}H_{12}O_7$

structure: III, R = H

weight:

- Chemical Abstract No.: 7241-98-7

- Synonyms:

  AFG_2 Dihydroaflatoxin G_1

  1H,12H-Furo(3',2':4,5)furo(2,3h)pyrano(3,4-c)(1)benzopyran-1,12-dione, 3,4,7a,9,10,10a-hexahydro-5-methoxy- (9CI)

- Molecular formula: $C_{17}H_{14}O_7$ structure: IV, R = H

  weight: 330.31

flatoxin M_1

Chemical Abstract No.: 6795-23-9

Synonyms

AFM_1 4-Hydroxyaflatoxin B_1

Cyclopenta(c)furo(3',2':4,5)furo(2,3-h)(1)benzopyran-1,11-dione, 2,3,6a,9a-tetrahydro-9a-hydroxy-4-methoxy- (9CI)

Molecular formula: $C_{17}H_{12}O_7$ structure: I, R_1 = OH, R_2 = CH_3

weight: 328.29

latoxin M_2

Chemical Abstract No.: 6885-57-0

Synonyms:

AFM_2 4-Hydroxyaflatoxin B_2

Cyclopenta(c)furo(3',2':4,5)furo(2,3-h)(1)benzopyran-1,11-dione, 2,3,6a,8,9,9a-hexahydro-9a-hydroxy-4-methoxy- (9C

Molecular formula: $C_{17}H_{14}O_7$ structure: II, R = OH

weight:

Aflatoxin P_1

1. Chemical Abstract No.: 32215-02-4

2. Synonyms:

   AFP_1

   Cyclopenta(c)furo(3',2':4,5)furo(2,3-h)(1)benzopyran-1,11-dione, 2,3,6a,9a-tetrahydro-4-hydroxy- (9CI)

3. Molecular formula: $C_{16}H_{10}O_6$

   structure: I, R_1 = H, R_2 = H

Other aflatoxins exist: for example, aflatoxin B_{2a} (Chemical Abstract No. 17878-54-5) has the same structure as aflatoxin B_2 except that an OH-group is at position 8 and aflatoxin G_{2a} (Chemical Abstract No. 20421-10-7) has the same structure as aflatoxin G_2 except that an OH-group is at position 9. Relatively little has been reported on these compounds.

Castegnaro et al. (1980) list data for the following metabolic products of aflatoxins:

1. Aflatoxin R_0 (aflatoxin F_1, aflatoxicol): reduction of cyclopentenone to the corresponding alcohol

2. Aflatoxin B_{2a} (aflatoxin B_1 hemiacetal, hydroxydihydroaflatoxin B_2)

3. Aflatoxin G_{2a} (9-hydroxyaflatoxin G_2)

4. Aflatoxin Q_1 (3-hydroxyaflatoxin B_1)

General Chemical and Physical Data

1. Density: No data.

2. Absorption spectroscopy: Naturally occurring aflatoxins and most of their identified metabolites exhibit three ultraviolet absorption maxima in the regions 214-226, 264-265, and 357-363 nm and fluorescence emission maxima at 425 or 450 nm. Individual values have been tabulated (IARC, 1976; Castegnaro et al., 1980).

3. Volatility: No data; may be considered nonvolatile.

4. Solubility: Slightly soluble in water (10-20 µg/ml); soluble in polar organic solvents such as ethanol, methanol, chloroform, dimethylsulfoxide. Aflatoxins are insoluble in nonpolar solvents, but stock and standard solutions in benzene, toluene, heptane, and cyclohexane containing a small amount of acetonitrile (Stubblefield, 1980) or n-propanol (Velasco, 1981) have been used.

5. Description, appearance: Pale yellow to colorless crystalline solids with fluorescence under UV light.

6. Boiling point: No data.

   Melting point: In the range of 237-293°C with decomposition. Individual values have been tabulated (IARC, 1976). The cited values are those of the naturally occurring (optically active) aflatoxins; the synthetic (racemic) compounds have lower meltin points.

7. Stability: Solid aflatoxins are stable if kept in the dark. Solutions are slowly decomposed (9-16% in 3 months) in the dark and decompose more rapidly when exposed to air and daylight or ultraviolet light; the extent varies with the solvent (Velasco, 1981). Chloroform solutions are reported to be stable for year when kept cold in the dark.

8. Chemical reactivity: The lactone ring is opened in strongly al- kaline solution; the vinyl ether double bond (AFB_1, AFG_1) is re- active towards oxidizing agents (hypochlorite, perborate, ozone

9. Flash point: No data.

10. Autoignition temperature: No data.

11. Explosive limits in air: No data.

Fire, Explosion, and Reactivity Hazard Data

1. Aflatoxins do not require special fire-fighting procedures or equipment and do not present unusual fire and explosion hazards. Because of the electrostatic nature of dry aflatoxins, fire fighters should wear full-face masks.

2. Conditions contributing to instability include exposure to heat and sunlight.

3. No incompatibilities have been reported.

4. Aflatoxins do not require nonspark equipment. When handled in flammable solvents, the precautions required for such solvents apply.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The Guide- lines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving AF.

1. Chemical inactivation: Validated methods have been reported (Castegnaro et al., 1980).

2. Decontamination: Turn off equipment that could be affected by AF or the materials used for cleanup. If more than 1 g has been spilled or if there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Wash surfaces with quantities of sodium hypochlorite solution. Glassware should be rinsed (in a hood) with methanol, followed by hypochlorite solution. Animal cages should be washed with hypochlorite solution. For details, see Castegnaro et al. (1980).

3. Disposal: It may be possible to decontaminate waste streams containing AF before disposal. For details, see Castegnaro et al. (1980). No waste streams containing AF shall be disposed of in sinks or general refuse. Surplus AF or chemical waste streams contaminated with AF shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing AF shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing AF shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with AF shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing AF shall be handled in accordance with the NIH radioactive waste disposal system.

4. Storage: Store solid materials and solutions in the cold and dark in sealed ampoules or amber screw-capped bottles or vials with Teflon cap liners. Avoid dispersal of electrostatically charged solid material while sampling.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: There are no reports on air or water sampling procedures. Various procedures have been described for sampling of agricultural commodities (Schuller et al., 1976).

2. Separation and analysis: The most commonly used procedures, as applied to food products, consist of extraction with organic solvents, separation of the extracts by column or TLC, and quantitation by densitometry or fluorescence spectroscopy. The official AOAC methods have been described (Horwitz, 1980), and solvent systems for the separation of various aflatoxins by TLC have been tabulated (Issaq and Cutchin, 1981). HPLC is the method of choice

at this time, particularly for the separation of aflatoxin metabolites from parent compounds or each other (Takahashi, 1977; Johnson et al., 1977; Colley and Neal, 1979). One application of these procedures to the analysis of aflatoxins in animal tissues has been published (Brown et al., 1973). Radioimmunoassay and enzyme-linked immunosorbent assay of aflatoxin M_1 in milk have the highest sensitivity (Pestka et al., 1981), and these methods show promise for application to other aflatoxins as well.

Biological Effects (Animal and Human)

1. Absorption: AFB_1 and AFG_1 are readily absorbed from the gastrointestinal tract in rodents and subhuman primates and from the respiratory tract in rats after intratracheal administration (Dickens et al., 1966).

2. Distribution: Oral and intraperitoneal aflatoxins or their metabolites are distributed to the liver and kidneys and probably to other organs.

3. Metabolism and excretion: Five distinct metabolic pathways for aflatoxins are known and are summarized with particular emphasis on AFB_1 (Campbell and Haynes, 1976; Colley and Neal, 1979). The first involves hydroxylation at position 4 (or position 9a in Chemical Abstracts nomenclature [refer to Section B for identification]), and the resulting products have been isolated from the milk of cows (Allcroft et al., 1966; Carnaghan et al., 1963) and urine of sheep (Holzapfel et al., 1966) fed aflatoxin-contaminated feed. Thus, $AFB_1 \rightarrow AFM_1$ and $AFB_2 \rightarrow AFM_2$. The second is O-demethylation (for instance, $AFB_1 \rightarrow AFP_1$), and the resultant metabolite was isolated from the urine of monkeys dosed with AFB_1 (Dalezios et al., 1971) as the sulfate or glucuronide conjugation compound. The third pathway, so far documented only for AFB_1, is a reduction of the cyclopentanone function to "aflatoxicol," and this metabolite was identified as the major one in Sprague-Dawley rats though not in mice and monkeys, which are resistant to AFB_1-induced carcinogenesis (Wong and Hsieh, 1978). The fourth pathway consists of oxidation at the cyclopentenone ring to produce AFQ_1, which is produced *in vitro* by monkey and human liver microsomes and is a urinary excretion product in the monkey (Campbell and Haynes, 1976). The fifth pathway, and perhaps the most important one in terms of carcinogenicity, consists of oxidation at the 2,3 (Chem Abstract 8,9) positions of the vinyl ether double bond through the (so far only postulated) 2,3-epoxide to 2,3-dihydro-2,3-dihydroxy AFB_1. This compound has been detected as a urinary metabolite in the form of its 2-glutathionyl derivative in rats (Degen and Neumann, 1978) and is the hydrolysis product of the reaction of AFB_1 with RNA in rat and hamster liver microsomes (Swenson et al., 1974) and with DNA. Both the epoxide and aflatoxicol (Nixon et al., 1981) have been postulated to be proximate or ultimate carcinogens metabolically derived from AFB_1. Excretion is in bile, urine, and milk in unchanged form (Dann et al., 1972) or in the form of metabolites as indicated above.

4. Toxic effects: The oral LD50s of AFB_1 are 0.55, 0.62, 2.0, 2.2, 5.5, 9.0, and 10.2 mg/kg in the cat, pig, guinea pig, monkey, rat mouse, and hamster, respectively. The only comparative toxicity studies with other aflatoxins have been carried out in day-old ducklings; oral LD50s for AFB_1, AFB_2, AFG_1, and AFG_2 are 18.2, 14.8, 39.2, and 172.5 µg per 50-g duckling (Carnaghan et al., 1963) and 16.6 and 62 µg per duckling for AFM_1 and AFM_2 (Holzapfel et al., 1966). These results indicate that metabolism by hydroxylation at the R or R_1 position does not materially alter toxicity and the less saturated compounds (structures I and III) are substantially more toxic than the others. Acute toxic effects of oral AFB_1 are on the digestive tract (emesis, anorexia); liver (jaundice, fatty degeneration, and necrosis); kidney (fatty degeneration); and central nervous system (coma, cerebral edema) in monkeys.

5. Carcinogenic effects: a. Animals. The most susceptible organ to oral AFB_1 is the liver (hepatocellular carcinomas in the rat and rhesus monkey). Other targets are kidney and colon. Inhalation of a mixture of AFB_1 and AFG_2 results in lymphatic leukemias. Feeding of several aflatoxins to pregnant rats results in hepatic carcinomas in the offspring. For a compilation of carcinogenic effects in animals, see IARC (1976).

   b. Humans. It has been generally assumed from epidemiological studies in African and Asian countries that there is a positive correlation between exposure to aflatoxin-contaminated foodstuffs and incidence of hepatitis, generalized hepatotoxicity, and incidence of hepatocellular carcinoma. In recent years, the role of aflatoxins as the direct causative agent in these outbreaks of hepatocarcinomas has been questioned, since equally good correlations with exposure to hepatitis B virus can be claimed. Aflatoxins may act primarily as immunosuppressive agents, causing an increase in hepatitis B virus carriers (Lutwick, 1979; Franco et al., 1982).

6. Mutagenic and teratogenic effects: AFB_1, AFB_2, AFG_1, and AFM_1 are mutagenic in the Ames test. AFB_1 is a potent teratogen in hamsters and rats.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes.

2. Ingestion: Drink plenty of water. Induce vomiting.

3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.

4. Refer to physician promptly. Undiluted bleach (5-6% NaOCl) or a mixture of sodium perborate and detergent, followed by soap and water, may be used for skin exposure. Gargles with a water solution of 1% sodium perborate and 1% sodium bicarbonate or a dentrifrice containing perborate may also be used for ingestion.

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